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Reczko, Eva. 1957. Electron microscopic investigations on the virus of Stomatitis papulosa (Elektronenmikroskopische Untersuchungen am Virus der Stomatitis papulosa). Central Book for Bacteriology, Parasitology, Infectious Diseases, and Hygiene (Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskranheiten und Hygien) 169: 425-433, 1957.

Stomatits papulosa is a relatively mild, contagious disease of bovines, first documented in 1936 by Ostertag and Bucca(1). These authors speculated that the causative agent was a filterable virus. Schraaf, Fraub and Beller (2) in 1937 observed changes in the mucosal lining of the mouth of experimental cattle being held for investigations on Trichomonas disease. These changes appeared to be traceable to the same virus. In their principal investigations, they determined, among other things, that the filterable agent was strongly host- and organ-specific. The induced infection appeared only in the mucosal lining of the mouths of cattle. In some cases, the virus could be detected in the blood. The appearance of papules on the feet was not observed. The histological picture of the mucosal lining of the mouth in the area of the papules showed a widening of the epithelial coat around the area of the papules. Ballooning degeneration of the cells of the stratum spinosum and exaggeration of the cells of the granular layer were noticed. The authors found multi-shaped acidophilic inclusion bodies in the ballooning cells. They were located either in the plasma or in the hollow spaces formed by atrophy of the protoplasm, and they often resembled a granular structure. On the basis of the stimated volume of viral particles, from an intermediate filtration, the kinds of cell inclusions, and the histological changes in the epithelium of the mouth, the authors discussed the possibility that the etiological agent is closely related to the pox virus group.

The problem of whether or not this was a pseudo-hoof and mouth disease occurring in cattle in South Africa, identical to Stomatits papulosa, and reported on by Bekker, Du Torr, and Quinlan (3), by De Kock, Du Torr, and Neitz (4) and by De Kock (5) was not clear until now.

Since Stomatitis papulosa is of less economic importance, it was given little consideration until now. Only recently, since it was noticed frequently in connection with epidemic outbreaks that produced severe economic losses in America, in Morocco, and in northern and central Germany, has more interest been given to it. Wagener (6) succeeded with cases occurring in northern Germany in identifying them as that disease which had been described in American as X-sickness or hyperkeratosis. The question concerns poisoning by application of chlrnaphthalene-containing industrial products, among which wood preservatives and tar- and asphal-containing products play a role. The last report concerning such a case came in 1954-55 from central Germany where ropes prepared with such materials were the cause of heavy damage among cattle herds. In all of the reports concerning the cases of poisoning, changes in the oral cavity characteristics of Stomatitis papulosa were mentioned. Olsen and Palionis (7) expressed the opinion that a virus-like agent such as that associated with Stomatitis papulosa is involved. In their work, they showed a ligh microscope picture of the epidermis of an experimentally infected animal that shows characteristics of Stomatitis papulosa and gives the impression that inclusion bodies also are present. Through administration of chlornaphthalene,

the authors were able to make experimental animals more susceptible to infection. From cases observed in central Germany, Heinig (8) ascertained that the causative agent of the stomatitis was filterable through A Seitz filter and that is could be transmitted from normal cattle independently of chlornaphthalene administration. Heinig(8), Beer (9), as well as Dedie and Mitrab (10) described the acidophilic bodies seen by Schaaf, Traub and Zeller. Pallaske (11) found that, on account of the reduction in resistance of the animals, the papulous changes show a strong tendency to spread and only a slight tendency to heal. Also, in these instances following a less mild case with proliferating papule formation, he could usually demonstrate acidophilic inclusion bodies.

With regards to chlornaphthalene poisoning, it has been observed that the virus of Stomatitis papulosa obviously appears much more frequently than was previously assumed. The breakdown of resistance might be the reason for the appearance of clinical manifestations.

Early in 1957, Stomatitis papulosa appeared enzemically in cattle herds in Badens-Wurttembergs, particularly in Handerstallen. These cases provided the opportunity to investigate electron microscopically the papulous changes in the mucous membranes of the mouth using ultra-thin sections and in spot preparations.

MATERIALS AND METHODS

Production of Material: The virus were obtained from a spontaneous infection of 6-8 month old cattle in a stable in Goppingen, in which all the animals there were involved. Calves were infected experimentally

for the study of ultra-thin sections of diseased-damaged tissues. From the original material, a portion of papulous mucous membrane, a 20 % emulsion in phosphate buffered isotonic saline (pH 7.4) was prepared. Infection occurred on the gingiva of the upper and lower jaw as a result of streak inoculations with the supernatant obtained by centrifugation of this material. On the second day, a reddening could be detected; on the third day, fully developed papules had appeared on the inoculation streaks. At this time, a small strip of papulous membrane was incised from the live animal.

Fixing and Embedding: 1 mm pieces of the material was immediately placed in 5 % phosphate-buffered formalin solution, pH 7.4. The next day, the specimens were transferred through multiple fresh saline solutions for two hours, left over night in 70 % ethanol, then placed for three hours in a solution of 1 % phosphotungstic acid and 70 % ethanol. They were then dehydrated as usual and embedded in butylmethyl acrylate. The ultrathin sections were prepared with a Sjostrand microtone and collected in 20 % alcohol.

Spot Preparations: The preparation of spot preparations followed the method described by Herzberg and Kleinschmidt (12). A fresh vertical section was lightly spotted on a coated screen. After a quick washing with water followed by air drying, it was coated with platinum-rhodium at a 21.8° angle.

Sections and spot preparations were examined with the Siemans Elmiscope No. 1. For the preparation of photomicrographs, Kranz-Contrast Ortho plates were used.

DISCUSSION OF PICTURES

The first three pictures are light microscope photographs of paraffin sections (hematoxilin-Eosin stain). Picture No. 1 is of the epithelial mucosa of the mouth of a healthy calf. The second picture is of a section through the same area, and shows that the tissue is from a calf infected with Stomatitis papulosa. In this picture, ballooned cells of the stratum spinosum are seen along with inclusion bodies. Picture No. 3 is an enlargement of the same area.

In Picture 4, an electron microscope photograph of an epithelial cell from papulous tissue, magnified 5000 times, the same changes can be seen as in Pictures 2 and 3. The cell boundries look like arked lines, whose sharp apexes are part of the intercellular bridges. Above and on the left side of the cell, it seems to be discontinous. Underneath, the cohesion to the neighboring cell is dissolved, and there is a broad intracellular gap. In part of the remainder of the enclosed cytoplasm in a clear empty space lies the shrunken nucleus with two round (slightly) thick structures in its center that are possibly swollen nuclear bodies. In the same space, adjacent to the nucleus, lies a structure that has somewhat the same radius as the nucleus and which undoubtedly is identical to the eosinophilic neoplasms referred to in the light microscope preparations as included bodies. Small, irregularly formed islands, which seem to consist of the same material, are seen to the right of the nucleus. On the inside of the inclusion bodies as well as on their borders lie ovoid-shaped particles about 200 mμ large. A large number of ovoid particles are bound into a group with an irregular arrangement and somewhat withdrawn. In the enlargements

it can be seen immediately that a smaller number of round particles lie within the inclusion bodies, and that these are surrounded by a membrane and have the same size as the ovoid particles. In Picture No. 6, two inclusion bodies with the same environment can be seen. On the left and lying underneath, are present only narrow round particles, which contained in a cavity like the ovoid particles. In the interior of the right side lie two inclusion bodies with irregularly formed, centered condensations whose structures appear to be coarse at this magnification. Many of these were found in inclusion bodies.

Pictures 7 and 8 show portions of inclusion bodies magnified 60,000 times. The focal condensations appear in both pictures. They show up distinctly from the surrounding loose and foamy material which makes up the great proportion of the inclusion bodies. Numerous small, but also extended foci of this type were also observed. In the enlarged picture, an irregular net-like structure can be seen which has a coarse granular appearance through the thick junctions of the network. In the rounded portions, where these junctions intersect, such as in the center, numerous, round bud-like porjections are found. They are enclosed by a membrane that is discontinous where it meets with the edge of the thickened center. These bud-like projections, about 200 mμ large, and rounded, are found inside the foamy materials of the inclusion bodies. They are distinquished from the foamy material in this way only - they are bound on one side with the thickened, granular focal condensation. In Picture No. 8, it can be seen on the left side that this union can also consist of only a narrow, filamentous bridge.

The previously mentioned round and ovoid particles, which could be viral particles or, very possibly, degrees of development of viral particles, form a very characteristic layer in relation to the inclusion bodies.

Inside the foamy material of the inclusion bodies only the round particles are found. Pictures 9a and 9b show characteristic particles of this kind. They have a thick outer inner body with a diameter of about 140 mμ and are surrounded by a membrane which seems to be linked in aproximately 33 loose double-walled chains. The entire structure has a diameter of about 207 mμ. The space between the inner body and the membrane is full of a material that has the same thickness as a structure similar to that of the foamy material of the the inclusion bodies. In many particles, fine filaments can be seen radiating from the inner body to the membrane. The slightly ovoid structure of the particle in Picture 9b is probably due to the pressure of the cutting tool. The intersice between the membrane and the inner body is often wider on one side (Pictures 9c and 10), and sometimes the membrane is not completely closed (Picture 10). Whether this form is due to the use of cutting tools or is naturally produced is a question for further research. Likewise, within the foamy material of the inclusion bodies, membranes are also formed in which the inner body is missing and which seems to include only some of the material of the surrounding environment (pictures 9d and 11).

The ovoid particles have some distinguishing features. They are 190 to 240 mμ long and 90 to 120 mμ wide. They lie only in the foamy material of the inclusion bodies. The impression is given that the [illegible] material was displaced by them or rolled itself away from them. Where they are found in the inclusion bodies, they are surrounded by an open spaces (Pictures 4-6). The ovoid particles appear mainly on the edge of the inclusion bodies, individually or in large groups in the free spaces within the cytoplasm and isolated within the degenerated cytoplasm. In relatively thinner sections and with stronger magnifications, it can be seen that not all of the ovoid particles have the same inner structure There appears to be a flowing transition between the different forms. The particles in Picture 9b have a relatively simple structure. The small part at the bottom left seems to be cut transversely to its longitudinal axis. It is surrounded by a 4-8 mμ membranelle which is attached from the inside out a layer of somewhat similar width and much less density. Left of it and at the right at two places, both of these layers are interupted by disintegration. Starting from the inside and going out, progressively wider layers of 3, 5, to 7 mμ in width are found which surround an oblong, slightly thicker body. Both of the particles lying in the middle of the picture and having been cut along their longitudinal axis, show a similar structure. Also, their various layers lie bound together around a long inner body. In many of the ovoid particles, the edge appears slightly corrugated or it appears as indentations in many cases. Picture 9f shows a particle exhibiting a number of striated bands of different widths which appear to transverse the particle. Investigations are under way to determine whether or not these are the result of successive developments, such as decomposition

Here and there in this material appear thickenings which have a net-like structure with wide points of junction and seem to separate the inclusion bodies of the round viral particles. Between the inclusion bodies and the membrane lies a layer displaying a structure similar to the foam-like material of the surroundings. Although distinctions exist, there is the question of similar developmen between this system and that observed with fowl pox by Morgan and co-workers (13) who studied cross-sections from infected chorioallantoic membranes. The material labeled by these authors as "Pyiroplasma" had a loose, foam-like composition like that of Stomatitis papulosa. The latter authors noted with Vaccinia and fowl pox viral particles a double membrane and a less thickened inner portion lying near the edge of the cytoplasm as well as extracellularly. These forms were considered by them to be a later developmental stage of viruses. Bernhard and coworkers (14) have made similar observations in their investigations of the virus of rabbit fibrosis, and Bauer and Constantin (15) were able to determine in systematic studies on infected tissue cultures that the premature forms, which turned into inclusion bodies after only six hours, are themselves put together from viroplasma and are surrounded by a simple membrane. After 8 days, in comparison, they found fibrosis viral particles that possessed a double membrane and showed a clearing in the center. It has been assumed that the free particles of Stomatitis papulosa represent a later stage of development.

In unfixed wet spot preparation that were prepared directly from infected tissue, only the free oval forms were found. These particles were generally 20 mμ larger than those usually found - a difference which may explain the different results with original material. While the canary pox virus prepared with these methods by Horsfall and Kleinschmidt (12) had a square shape, these particles retained their oval shape. This points out that the virus of Stomatitis papulosa is not of the square virus form.

The observations presented here on thin sections of fully developed papules have shown that the elementary bodies of the Stomatitis papulosa virus appear in different forms, from which it must be assumed that the question concerns successive stages of development of virus particles. The evidence for the correctness of these assumptions will have to be produced through additional investigations of disease altered tissue at different stages of the disease.

SUMMARY

The included bodies, demonstrated in the case of bovine Stomatitis papulosa were examined in the electron microscope in ultra-thin sections.

The material of which they mainly consist is loose and foamlike and contains focal consolidations.

Included within this foamlike material, round particles are found with a dense interior body, a surrounding membrane and a less dense layer, filling the interspace. These particles have a diameter of about 207 mμ. The existence of incomplete particles of this kind offers the

conclusion due to their special form and situation, that they are build from material of the included bodies. The author presumed, that these particles are virus particles. Other ovular particles, which are approximately 215 mμ long and 105 mμ wide, are considered as virus particles in a different (letter?) stage of development. They are not bedded into the material of the included bodies, but lie freely in great numbers in its hollow space, on its outer margin, within the intercellular gaps and single ones also within the cytoplasm.

These particles were also found in steamed dab-preparations. Contrarily to the virus of canary-small pox, which is of square structure when demonstrated according to this method, these particles kept their ovular form, ~~thereby undercining the~~ presumption, that the virus of Stomatitis papulosa, according to Findeisen, must not be counted among the square types of virus.

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